Level of Glycogen Stores and Amount of Ingested Glucose Regulate Net Carbohydrate Storage by Different Mechanisms

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The respective effects of the level of glycogen stores and the size of the glucose load on the pathways of carbohydrate (CHO) metabolism were compared over the 5-hour period following glucose ingestion in normal human subjects. For this purpose, labeling of the oral glucose load with [3-^{3}H]- and [U^{14}C] glucose was combined with indirect calorimetry. In group I, 75 g glucose was given to subjects who had either been "fed" with intravenous (IV) glucose or fasted for 13, 24, or 36 hours, or 4 days. In fed versus 4-day–fasted subjects, net CHO storage averaged approximately 15 versus 63 g/5 h ($P < .001$). About 83% of the increase in fasted subjects was due to suppression of glycogen breakdown, with only minimal stimulation of glycogen synthesis from oral glucose. Over the whole range of nutritional conditions tested, a strong positive correlation existed between basal CHO oxidation and glycogen breakdown occurring during the oral glucose tolerance test (OGTT), suggesting that the initial degree of repletion of hepatic glycogen stores is a major determinant of postprandial glycogen turnover. In group II, OGTTs with 33 and 100 g glucose were compared in 13-hour–fasted subjects. Net storage rose from approximately −6 to ~ 37 g/5 h ($P < .001$) solely because of an increase in glycogen synthesis with no inhibition of glycogen turnover. Overall, these results show that the initial dietary state and the size of the glucose load modulate postprandial net CHO accumulation by different mechanisms.

AN ESSENTIAL FUNCTION of dietary carbohydrate (CHO) consumption is to restore adequate glycogen stores in liver and muscle. Among others, 2 important factors regulate whole body net glycogen retention after a CHO meal: (1) the size of the oral load, and (2) the level of initial glycogen stores, which depend mainly on the composition of the usual diet, the duration of the fast and the degree of physical activity preceding the glucose challenge. Net CHO accumulation after oral glucose depends on the balance between the synthesis of glycogen from the oral load and endogenous glycogen mobilization, which is known to persist during the postprandial period.[1,2] However, the relative contribution of each of these processes to regulating postprandial glycogen retention are not yet clear.

In the present study, which extends our previous work,[2] separate estimations of glycogen synthesis and the degradation of whole body glycogen were obtained during an oral glucose tolerance test (OGTT) by addition to the glucose load of [U-^{14}C]- and [3-^{3}H]glucose combined with indirect calorimetry, under 2 main experimental settings: first, postprandial glucose metabolism was explored over a broad range of nutritional conditions extending from the fed state to a total 4-day fast with several intermediate periods of fasting. Second, we examined the impact of the size of the load (33 *v* 100 g) on the metabolic partition of oral glucose in overnight fasted subjects. The results indicate that nutritional conditions and the size of the glucose load respectively modulate net postprandial glycogen retention by different mechanisms.

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MATERIALS AND METHODS

Protocol

Twenty normal volunteers with no history of diabetes participated in the study. Their characteristics were the following: age, 27 ± 1 years; gender, 4 females and 16 males; body weight, 72 ± 3 kg; and body mass index, 22.5 ± 0.6 kg/m^2. The nature, purpose, and potential risks of the study were explained to the subjects and their written informed consent was obtained before participation. The protocol was approved by the Ethics Committee of the Faculty of Medicine of Brussels Free University. Subjects were divided into 2 groups.

Group I (effect of nutritional conditions prior to glucose ingestion). Fourteen normal volunteers underwent a total of 40 OGTTs performed after 1 of 4 periods of fasting (13 hours overnight, 24 or 36 hours, or 4 days) or in the "fed" state. The fed state was obtained as follows: on the day before the test, subjects ingested a last evening meal at 7 PM. It contained 133 g of CHO and was immediately followed by an overnight intravenous (IV) glucose infusion. Glucose was infused as a 20 % solution at the rate of 4 mg/kg · min for 11 hours, and the infusion was discontinued at 6 AM, 2 hours before the OGTT. The glucose concentration and CHO oxidation were measured after 5 hours of glucose infusion, and just before its discontinuation. The average total amount of glucose infused was 201 ± 13 g.

The allocation of OGTTs to fed and variously fasted subjects was such that at least 6 paired comparisons with the overnight fasted state were available for each of the other nutritional conditions. In each individual, the tests were performed in random order and were separated by at least 2 weeks.

For each OGTT, the 75 g of glucose used was labeled with [3-^{3}H]glucose (150 μCi) to measure glycolysis, and with [U-^{14}C]glucose (10 μCi) to measure glucose oxidation. Using the heated hand technique,[3] arterialized venous blood samples were collected during a basal period of 20 minutes at −20, −10, and 0 minutes, and every 30 minutes thereafter for 5 hours. Respiratory gas exchanges were measured during the basal period and for 15-minute periods every 30 minutes for the remaining 5 hours, using a Deltatrac Metabolic Monitor (Datex, Helsinki, Finland). Every hour after glucose ingestion, a sam-

ple of expired air was collected in a rubber bag for immediate measurement of CO_2 specific activity. Timed urine specimens were obtained before and at the end of the OGTT. In the case of fasted subjects, urine was collected on hydrochloric acid to prevent loss of ammonia. On a separate occasion, total body water volume was measured in each subject, using an IV bolus injection of ^3H_2O (20 μCi), as described previously.[4] The volume of extracellular fluid was assumed to constitute 33 % of total body water.[5] All isotopes were purchased from Dupont-NEN (Boston, MA).

Group II (effect of the size of the glucose load). Six other subjects were studied only in the overnight fasted state (13-hour fast). Each of them underwent two OGTTs in random order at an interval of at least 2 weeks, one with 33 g of glucose and the other with 100 g. Otherwise the glucose tests, including glucose labeling, were carried out as for group I.

Analytical Procedures

Blood samples were collected in heparinized syringes and transferred to tubes kept on ice. The samples used to measure unlabeled and labeled glucose and lactate concentrations contained NaF. After centrifugation at 4°C, plasma was stored at –20°C until assay. Plasma glucose was determined by a glucose oxidase method (Test Combination Glucose; Boehringer Mannheim, Mannheim, Germany). Plasma [^{3}H]glucose and ^3H_2O were determined after deproteinization by the Somogyi method. [^{3}H]glucose was counted by dual scintillation spectrometry on evaporated filtrates reconstituted with water, and ^3H_2O was determined as the difference between the tritium counts obtained with and without evaporation. ^3H_2O in plasma water was calculated by dividing its concentration in total plasma by 0.93. Lactate and 3-hydroxybutyrate (3-OHB) were determined on a neutralized perchloric filtrate of plasma by standard enzymatic methods.[6] Plasma concentrations of free fatty acids (FFA) (NEFA; Wacko, Neuss, Germany) and urea (Urea Merckotest; Merck, Darmstad, Germany) were determined with enzymic methods. The levels of plasma insulin (Pharmacia Insulin RIA, Pharmacia & Upjohn Diagnostics, Uppsala, Sweden) and glucagon (Glucagon RIA kit, Linco, St. Charles, MO) were determined by radioimmunoassay (RIA). Total urinary nitrogen was assayed by the Kjeldhal method[7] with a Kjeltec 1 apparatus (Tecator, Höganäs, Sweden). $^{14}CO_2$ specific activity in expired air was determined as described previously.[2] All determinations were made in duplicate.

Calculations

Calculation of the rates of carbohydrate (CHO) disposal in various pathways using a combination of isotopes and indirect calorimetry during exogenous glucose administration have already been indicated and their significance and limitations fully discussed in previous reports.[2, 4] They are briefly summarized below.

Isotope data. The pathways of oral glucose metabolism were calculated only for the entire 5-hour period, at the end of which it was assumed that the oral load had been completely absorbed by the gut.[8] Because the 2 tracers ([^{3}H]- and [^{14}C]glucose were incorporated into the ingested glucose, no information was obtained about either hepatic glucose output or the systemic appearance rate of oral glucose. Calculations were made stepwise, as follows: at the end of the 5-hour period, the amount of oral glucose remaining in the extracellular fluid was calculated as the ratio of the extracellular [^{3}H]glucose pool to the [^{3}H] specific activity of the glucose load. Oral glucose uptake by tissues was calculated as the difference between the amount of ingested glucose and the residual extracellular glucose pool of oral origin. Glycolysis was calculated[9] as the ratio of the amount of ^3H_2O accumulated in total body water at 5 hours (plus the ^3H_2O lost in urine) to the [^{3}H] specific activity of the load. Net glycogen synthesis by the direct pathway (glucose → glucose-1-P → glycogen) was calculated as oral glucose uptake – glycolysis.[4] Oral glucose oxidation was assessed as the ratio of cumulative $^{14}CO_2$ production over 5 hours to the [^{14}C] specific activity of the glucose drink. Results were divided by 0.71 to account for incomplete $^{14}CO_2$ recovery in expired air, as determined in a previous study under the specific conditions of an OGTT.[2] As shown in that study, this correction factor is not affected by a previous fast.

Nonoxidative oral glucose disposal was calculated as the difference between oral glucose uptake and isotopically determined glucose oxidation. It includes all nonoxidative pathways of oral glucose disposal, ie, glycogen synthesis, nonoxidative glycolysis, and net de novo lipogenesis. Because lactate was not significantly elevated above baseline at the fifth hour of the OGTT (data not shown), it was assumed that the extra lactate formed during the OGTT having escaped oxidation had been converted to glycogen by the end of the test. De novo lipogenesis has been shown to be a minor pathway, even in CHO-fed individuals.[10] Therefore, nonoxidative glucose disposal, calculated as specified above, should be approximately equivalent to net glycogen synthesis by both the direct and indirect pathways. The indirect pathway was computed as nonoxidative glycolysis.[4] It is acknowledged that the factor used to correct $^{14}CO_2$ data, and therefore the absolute figures for oral glucose oxidation and storage, are necessarily approximate. However, since the same factor was used under all experimental conditions, the comparison of test results should be valid.

Indirect calorimetry. CHO and lipid oxidation rates were calculated[11] from the $\dot{V}o_2$, $\dot{V}co_2$, and urinary nitrogen output corrected for the changes in the urea nitrogen pool.[12] The data for fasted subjects were also corrected for ketonuria and changes in the ketone body pool occurring during the OGTT,[11] assuming a functional volume of distribution of 0.2 L/kg body weight. Total CHO oxidation, measured by indirect calorimetry, includes the oxidation of both oral glucose and of glucose issued from endogenous glycogen breakdown, which is known to persist during exogenous glucose administration.[13] Therefore, unlabeled glycogen oxidation was calculated as total carbohydrate oxidation minus the oral glucose oxidation, as determined from $^{14}CO_2$ production.[14-17]

Net CHO balance was calculated as the difference between oral glucose uptake and total CHO oxidation. It is a measure of net glycogen accumulation in liver and muscle. For obvious arithmetic reasons, it is also equal to the difference between the glycogen synthesized from oral glucose (via direct plus indirect pathways) and unlabeled glycogen oxidation.

Values are means ± SE. Comparisons between subgroups concern the mean concentrations and fluxes for the basal period, and the mean concentrations and cumulative fluxes for the 0- to 5-hour posprandial period. These comparisons were made using a paired or unpaired *t* test, as required. In case of repeated measures (Fig 1), an analysis of variance (ANOVA) for 2 factors (group, time) with repeated measures on time was performed using SUPERANOVA (Abacus Concepts, Berkeley, CA). When the F ratio reached the level of significance ($P < .05$), pairwise comparisons of means were done using Scheffé's or Student's *t* test. Linear regression and covariance analysis were performed out by standard techniques. *P* values below .05 were considered significant.

RESULTS

Effect of Initial Nutritional Status on Oral Glucose Metabolism

The changes in basal metabolic parameters induced by the various periods of fasting and by prior glucose feeding are displayed in Table 1. As expected, fasting was associated with a fall in glucose and insulin concentrations and in CHO oxidation, and a rise in FFA, 3-OHB, and fat oxidation. Most of

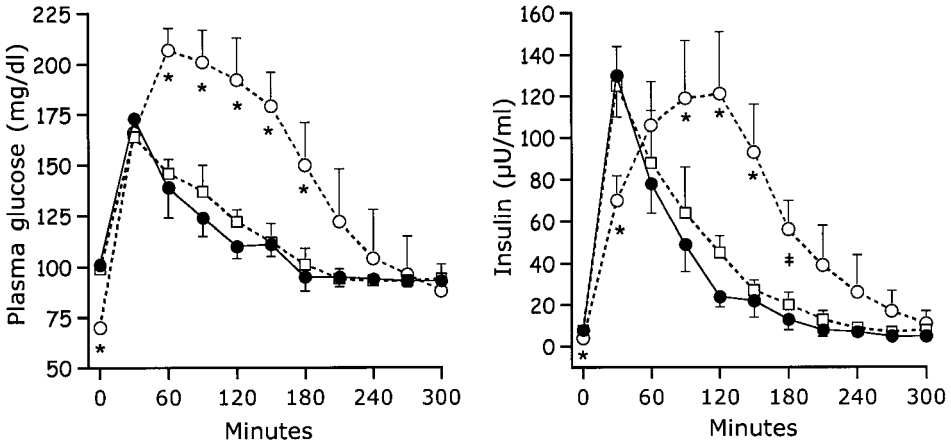

Fig 1. Glucose and insulin concentrations during an OGTT in 6 subjects studied after a 13-hour fast (●—●), a 4-day fast (○–○), and after prior "feeding" (□–□). Statistical significance of paired comparisons with the 13-hour fast: ‡$P < .05$; *$P < .001$ or less.

the differences are statistically significant, even for a short fast (eg, for 24 hours *v* 13). Note that the 4-day–fasted subjects were markedly ketotic. In these subjects, CHO oxidation was not significantly different from 0. In the fed subjects prepared by glucose loading, the overnight glucose concentrations respectively measured after 5 and 11 hours of glucose infusion averaged 125 ± 7 and 131 ± 10 mg/dL, and the corresponding CHO oxidation rates were 178 ± 7 and 234 ± 17 mg/min. Two hours after the end of the infusion, at the start of the OGTT, glucose, insulin, FFA and 3-OHB had returned to their normal ranges (Table 1), but CHO oxidation remained significantly elevated (39.3 ± 4.1 *v* 26.2 ± 4.5 g/5 h; $P < .02$). Protein oxidation was not significantly affected by the nutritional manipulations, except for a slight fall after 4 days of fasting.

The effects of increasing periods of fasting and of prior "feeding" on mean 0- to 5-hour substrate and hormone concentrations and glucose fluxes are shown in Table 2. The time courses of glucose and insulin concentrations in the 6 subjects studied after 13 hours and 4 days of fasting and after prior "feeding" are depicted in Fig. 1. The glycemic response was already augmented after 24 hours compared to 13 hours of fasting (Table 2). This was also the case for the mean insulin responses after 36 hours and 4 days of fasting, but the early insulin peaks tended to be blunted (Fig 1). Mean glucagon, FFA, and 3-OHB levels were elevated during the OGTTs performed after a fast of 4 days or less. Prior "feeding" did not affect any of these parameters.

Large changes in the pathways of glucose handling were observed after 4 days of fasting (Table 2). Tissue uptake of oral glucose was unchanged, but glycolysis and oxidation of oral glucose were both significantly inhibited. Conversion of glucose to glycogen by the direct pathway was stimulated (40 ± 3 *v* 31 ± 2 g/5 h; $P < .01$), but total conversion by the direct and indirect pathways combined only rose slightly (59 ± 1 *v* 54 ± 1 g/5 h; $P < .05$). Indirect calorimetry showed marked suppression of total CHO oxidation (7 ± 1 *v* 39 ± 3 g/5 h; $P < 0.001$) mainly due to the complete inhibition of glycogen oxidation (- 4 ± 1 *v* 22 ± 2 g/5 h; $P < .001$). The net CHO balance almost doubled (63 ± 1 *v* 32 ± 3 g/5 h; $P < .001$). Similar changes, but of smaller amplitude, were observed for shorter periods of fasting (36 and 24 hours, Table 2).

Prior feeding altered the metabolic response to OGTT in the opposite way to that observed after fasting. Glycolysis and total CHO oxidation rose significantly but oral glucose oxidation did not. Oral glucose conversion to glycogen decreased, both via the direct pathway and by the direct and indirect pathways

Table 1. Influence of the Duration of Fasting and of IV Glucose "Feeding" on Basal Parameters

	Duration of Fasting							
	13 h	24 h	13 h	36 h	13 h	4 days	13 h	"Fed"
Parameter	(n = 6)		(n = 7)		(n = 6)		(n = 6)	
Glucose (mg/dL)	97 ± 2	87 ± 3	98 ± 3	81 ± 1†	101 ± 2	70 ± 1*	101 ± 2	99 ± 2
Insulin (μU/mL)	8 ± 1	5 ± 1†	7 ± 1	4 ± 1†	8 ± 1	4 ± 1†	8 ± 1	8 ± 1
Glucagon (pg/mL)	61 ± 9	66 ± 13	60 ± 8	74 ± 12	65 ± 5	85 ± 16	65 ± 5	58 ± 8
FFA (mmol/L)	0.33 ± 0.03	0.74 ± 0.05*	0.39 ± 0.02	0.71 ± 0.09*	0.31 ± 0.03	0.84 ± 0.05*	0.31 ± 0.03	0.47 ± 0.06
3-OHB (mmol/L)	0.06 ± 0.02	0.45 ± 0.11†	0.09 ± 0.02	1.04 ± 0.23†	0.07 ± 0.04	4.75 ± 0.51*	0.07 ± 0.04	0.06 ± 0.02
ACAC + 3-OHB (mmol/L)	—	0.74 ± 0.15	—	1.47 ± 0.32	—	6.46 ± 0.58	—	—
CHO oxidation (g/5 h)	31.2 ± 4.1	18.2 ± 2.3†	31.0 ± 3.5	13.5 ± 3.5‡	26.2 ± 4.5	−2.6 ± 2.2*	26.2 ± 4.5	39.3 ± 4.1‡
Fat oxidation (g/5 h)	13.9 ± 1.5	21.6 ± 1.3*	14.2 ± 1.6	23.6 ± 1.6†	16.8 ± 2.7	33.0 ± 1.9*	16.8 ± 2.7	11.8 ± 2.4
Protein oxidation (g/5 h)	13.8 ± 1.9	9.4 ± 0.5	14.6 ± 2.0	10.5 ± 1.1	16.1 ± 0.7	12.7 ± 0.4†	16.1 ± 0.7	15.7 ± 1.1

NOTE. Data are means ± SE.

Statistical significance of pairwise comparisons with the 13-hour fast: *$P < .001$; †$P < .01$; ‡$P < .05$.

Abbreviations: FFA, free fatty acids; 3-OHB, 3-hydroxybutyrate; ACAC, acetoacetate; CHO, carbohydrate.

Table 2. Effect of the Duration of Fasting on the Metabolic Response to Oral Glucose

	Duration of Fasting							
	13 h	24 h	13 h	36 h	13 h	4 d	13 h	"Fed"
Parameter	(n = 6)		(n = 7)		(n = 6)		(n = 6)	
Concentrations								
Glucose (mg/dL)	116 ± 2	137 ± 5†	117 ± 2	130 ± 2†	113 ± 4	150 ± 15‡	113 ± 4	116 ± 2
Insulin (μU/mL)	27 ± 4	34 ± 4	26 ± 4	35 ± 3‡	34 ± 5	66 ± 14‡	34 ± 5	41 ± 6
Glucagon (pg/mL)	61 ± 10	60 ± 9	55 ± 6	62 ± 7‡	73 ± 10	92 ± 12‡	73 ± 10	58 ± 7
Lactate (mmol/L)	1.20 ± 0.08	1.24 ± 0.10	1.25 ± 0.05	1.33 ± 0.09	1.09 ± 0.04	1.06 ± 0.06	1.09 ± 0.04	1.25 ± 0.10
FFA (mmol/L)	0.27 ± 0.02	0.36 ± 0.03	0.30 ± 0.01	0.42 ± 0.05	0.29 ± 0.03	0.64 ± 0.02*	0.29 ± 0.03	0.24 ± 0.03
3-OHB (mmol/L)	0.08 ± 0.03	0.18 ± 0.04‡	0.09 ± 0.02	0.32 ± 0.03†	0.05 ± 0.02	1.75 ± 0.26†	0.05 ± 0.02	0.03 ± 0.02
ACAC 3-OHB (mmol/L)	—	0.30 ± 0.06	—	0.50 ± 0.06	—	2.77 ± 0.39	—	—
Fluxes (g/5 h)								
Oral glucose								
Uptake	71 ± 1	69 ± 1	71 ± 1	71 ± 1	71 ± 4	70 ± 1	71 ± 4	69 ± 5‡
Glycolysis	43 ± 2	39 ± 1	41 ± 2	38 ± 2	40 ± 2	30 ± 2*	40 ± 2	48 ± 1†
Storage (D)	28 ± 3	30 ± 1	30 ± 3	33 ± 2	31 ± 2	40 ± 3†	31 ± 2	21 ± 1*
Oxidation	20 ± 1	16 ± 1†	21 ± 1	17 ± 1*	17 ± 1	11 ± 1*	17 ± 1	18 ± 1
Storage (D + I)	51 ± 1	53 ± 1‡	50 ± 1	54 ± 1*	54 ± 1	59 ± 1‡	54 ± 1	51 ± 1‡
Total CHO oxidation	43 ± 2	31 ± 2†	44 ± 2	27 ± 4†	39 ± 3	7 ± 1*	39 ± 3	54 ± 3†
Net CHO balance	28 ± 2	39 ± 2†	27 ± 2	44 ± 4†	32 ± 3	63 ± 1*	32 ± 3	15 ± 3†
Glycogen oxidation	23 ± 2	15 ± 2‡	23 ± 2	10 ± 3‡	22 ± 2	−4 ± 1*	22 ± 2	36 ± 3*
Fat oxidation	14 ± 2	17 ± 2‡	13 ± 2	19 ± 2†	16 ± 2	29 ± 2*	16 ± 2	10 ± 2‡

NOTE. Concentrations are mean values, and fluxes are integrated data calculated for the 5 hours of the OGTTs.
Statistical significance of pairwise comparisons with the 13-hour fast: *$P < .001$; †$P < .01$; ‡$P < .05$.
Abbreviations: D and I, direct and indirect pathways of glycogen synthesis.

combined, but in the latter case the decrease was of borderline significance. Glycogen oxidation rose by approximately 64% (36 ± 3 *v* 22 ± 2 g/5 h; $P < .001$) and the net CHO balance fell markedly (15 ± 3 *v* 32 ± 3 g/5 g; $P < .01$).

All the individual CHO fluxes measured during the OGTTs in Group I are plotted in Fig 2 against basal CHO oxidation, which varied considerably from −10 to +52 g/5 h. Glycolysis and the oxidation of oral glucose, glycogen, and total CHOs,

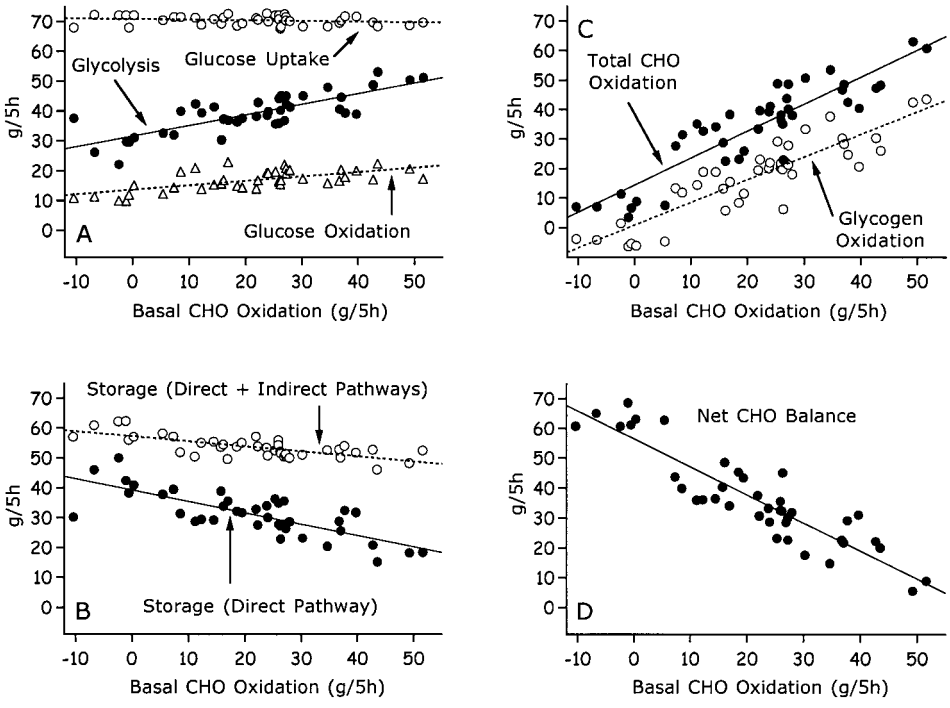

Fig 2. Relationship between basal CHO oxidation and oral glucose (A and B) or total CHO (C and D) fluxes following ingestion of 75 g glucose. Tests were performed under various nutritional conditions, ranging from a "fed" state to 4 days of fasting. $P < .001$ for all correlations except glucose uptake ($P > .05$).

Table 3. Comparison Between OGTTs With 33 and 100 g in 13-Hour–Fasted Subjects

Parameter	Basal		OGTT	
	33 g	100 g	33 g	100 g
Concentrations				
Glucose (mg/dL)	93 ± 1	93 ± 1	102 ± 3	114 ± 2‡
Insulin (μU/mL)	6 ± 1	6 ± 1	14 ± 1	38 ± 5†
Glucagon (pg/mL)	85 ± 7	87 ± 7	75 ± 6	77 ± 4
Lactate (mmol/L)	0.89 ± 0.12	0.83 ± 0.11	0.94 ± 0.07	1.23 ± 0.07†
FFA (mmol/L)	0.40 ± 0.05	0.40 ± 0.05	0.38 ± 0.07	0.24 ± 0.03‡
Fluxes (g/5 h)				
Oral glucose				
Uptake			31 ± 1	95 ± 1*
Glycolysis			22 ± 1	52 ± 1*
Storage (D)			9 ± 1	43 ± 2*
Oxidation			11 ± 1	26 ± 1*
Storage (D + I)			20 ± 1	69 ± 1*
Total CHO oxidation	33 ± 7	34 ± 6	37 ± 6	59 ± 4*
Net CHO balance			−6 ± 6	37 ± 5*
Glycogen oxidation			26 ± 5	33 ± 4
Fat oxidation	13 ± 3	16 ± 4	17 ± 2	9 ± 2*

NOTE. For the OGTT period, concentrations were averaged and fluxes integrated over 5 hours.
Statistical significance of pairwise comparisons between 33-g and 100-g tests: *$P < .001$; †$P < .01$; ‡$P < .05$.

correlated positively with basal CHO oxidation ($P < .001$, Fig 2A and C), whereas an inverse relationship ($P < .001$) was observed for glucose storage estimated by isotope techniques (Fig 2B) or by indirect calorimetry (Fig 2D).

Effect of the Size of the Glucose Load (33 v 100 g) on Oral Glucose Metabolism

The 6 subjects in group II tested with both 33 and 100 g glucose had comparable basal metabolic characteristics for each test (Table 3). As expected, the 100-g test was associated with higher mean plasma glucose, insulin and lactate levels and lower FFA concentrations. Oral glucose uptake rose in proportion to the glucose load. All the pathways of glucose metabolism were stimulated. This was particularly obvious for the net CHO balance, which was not significantly different from zero during the OGTT with ingestion of 33 g of glucose but averaged 37 ± 5 g/5 h during the test with 100 g. This effect was due to strong stimulation of glycogen synthesis, both in absolute terms (69 ± 1 *v* 20 ± 1 g/5 h; $P < .001$) and relative to glucose uptake (45 ± 2 *v* 29 ± 3%; $P < .005$ for the direct pathway and 73 ± 1 *v* 63 ± 2%, $P < .005$ for the direct and indirect pathways combined). Note that glycogen oxidation was not significantly affected by the size of the glucose load (33 ± 4 *v* 26 ± 5 g/5 h; $P > .05$).

Comparison between Figs 2 and 3 shows that the relationships between basal CHO oxidation and the rates of glucose metabolism in the various pathways that were observed with the 75 g load (Fig 2) also applied to the 33- and 100-g loads (Fig 3), although in the latter cases several correlations were not statistically significant due to the small number of individuals tested. Figure 3E shows that unlabeled glycogen oxidation during the 33- and 100-g OGTTs was positively related to basal CHO oxidation ($P \leq .001$) with no significant difference in the slopes of the 2 regression lines. The level of the regression line was slightly but significantly more elevated ($P < .01$) with the 100-g load.

DISCUSSION

This study was designed to explore whole body metabolic fate of oral glucose with special reference to storage and to establish how it is affected by the degree of previous fasting (group I) and the size of the glucose load (group II).

Group I

In overnight fasted subjects, who had ingested 75 g glucose, approximately 71 g was taken up by tissues within 5 hours. The isotope data indicate that approximately 20 g was oxidized and approximately 51 g disposed of nonoxidatively and thus presumably converted to glycogen in muscle and liver. However, the net glycogen balance calculated from the calorimetry data amounted to only approximately 28 g, suggesting that approximately 23 g of glycogen was mobilized from pre-existing unlabeled stores, thus confirming the existence of a glycogen turnover under conditions of postprandial net glycogen synthesis.[1,2,13]

Compared to the overnight fasted state, prolonged fasting (4 days) induced major metabolic changes, both in the basal state (Table 1) and after glucose ingestion (Table 2). Basal CHO oxidation stopped completely, lipid oxidation more than doubled and total ketone body concentrations averaged approximately 6.5 mmol/L, indicating that the fast was well observed. Glucose tolerance was reduced indicating a state of "starvation diabetes."[3] The early insulin response was blunted (Fig 1) but the late response was amplified in the presence of more elevated glucose concentrations, these observations being compatible with the well-known state of insulin resistance induced by fasting.[4] Oral glucose uptake was unchanged but the net CHO balance almost doubled (63 *v* 32 g/5 h; $P < .001$) due to the

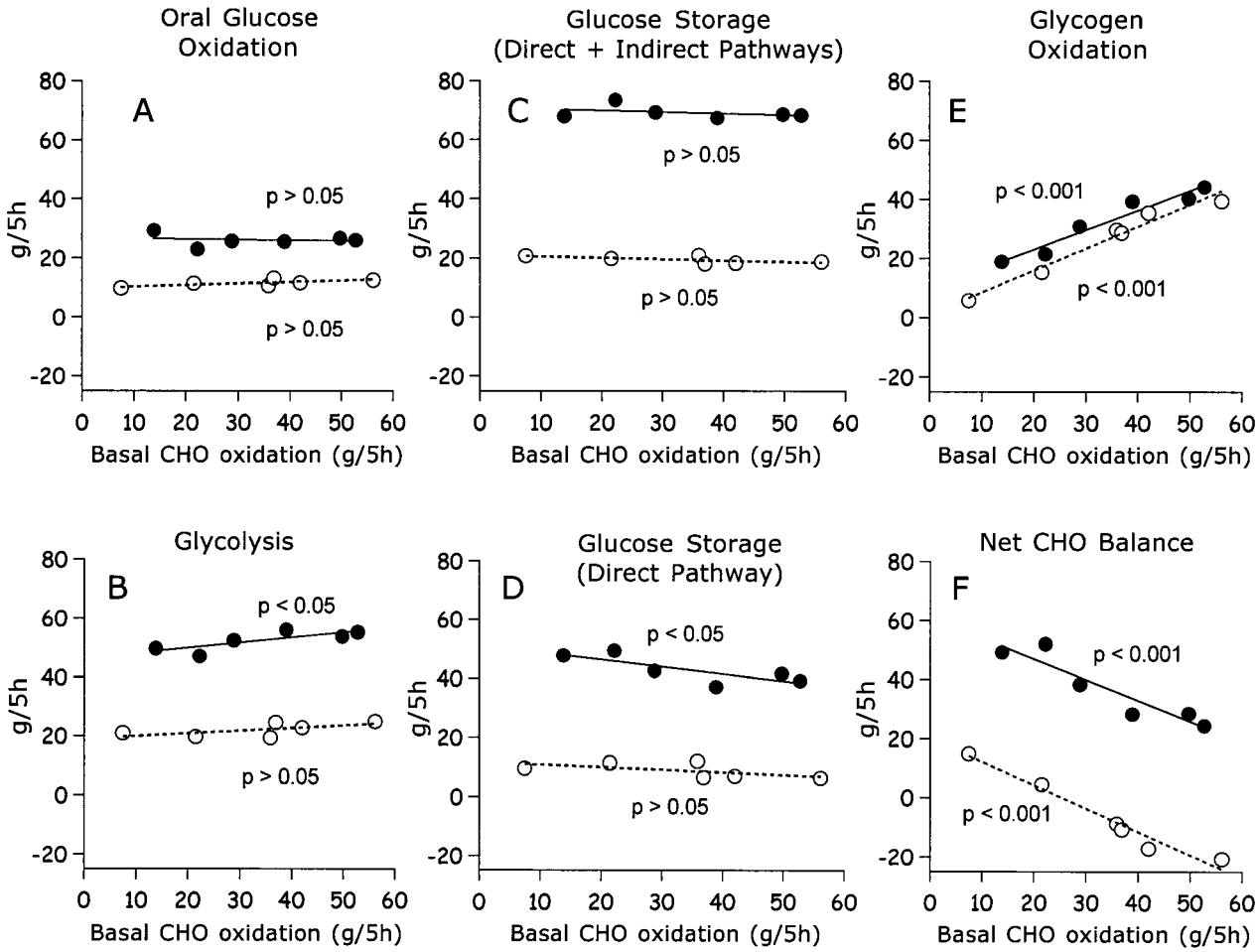

Fig 3. Relationship between basal CHO oxidation and oral glucose (A–D) or total CHO (E and F) fluxes, following ingestion of 33 g glucose (○–○) or 100 g glucose (●—●) in overnight fasted subjects.

total suppression of unlabeled glycogen breakdown (−4 *v* 22 g/5 h; $P < .001$) with only a minor stimulation of the synthesis of glycogen from oral glucose (59 *v* 54 g/5 h; $P < .02$). The stimulation of CHO retention by fasting is a very sensitive process, as documented by the fact that simply extending the fast from 13 hours to 24 hours was sufficient to stimulate net glycogen accumulation during an OGTT by approximately 39%, again mostly by virtue of an inhibition of unlabeled glycogen breakdown.

Changes in opposite directions were recorded in "fed" subjects and in this case too, the changes in oral glucose metabolism were relatively moderate compared to those observed in total CHO oxidation and net CHO balance. Thus, about 80% of the observed decrease in CHO balance related to prior feeding was due to the stimulation of unlabeled glycogen breakdown and only 20% to a reduction in the conversion of oral glucose to glycogen.

It is not quite appropriate to compare subgroups of subjects on the basis of the duration of food deprivation because the latter does not accurately predict the degree of "metabolic" fasting, which is much better reflected by the rate of basal CHO oxidation. This parameter, which exhibited large individual variations in each subgroup, reflects mainly hepatic glycogen oxidation[15] and should probably therefore be closely related to preprandial hepatic glycogen content. In accordance with these considerations, we analyzed in Fig 2, the correlations between individual postprandial CHO fluxes and basal CHO oxidation over the entire spectrum of nutritional conditions tested. For the individuals who fasted longest with low or even slightly negative basal CHO oxidation (left side of the abscissa) net glycogen accumulation (Fig 2D) amounted to 60 to 70 g/5 h representing 90% or more of the oral glucose uptake, whereas in the most fed individuals (right side of the abscissa), basal CHO oxidation exceeded 50 g/5 h and the net glycogen balance did not exceed 10 g/5 h, ie, less than 15% of glucose uptake. Because oral glucose uptake over 5 hours was not related to initial nutritional conditions (Fig 2A), modulation of the net CHO balance depended entirely on total CHO oxidation, which correlated positively with basal CHO oxidation (Fig 2C). The variations in total CHO oxidation themselves were much more dependent on

the rate of unlabeled glycogen oxidation (slope 0.771; Fig 2C) than on that of oral glucose oxidation (slope 0.147; Fig 2A).

In theory, the modulation of post-glucose CHO fluxes by nutritional conditions might depend on 3 main factors: (1) the initial level of glycogen stores; (2) the FFA concentrations and fat oxidation rates, and (3) the hormonal and glycemic responses to glucose ingestion.

The initial level of glycogen stores, as reflected by basal CHO oxidation, emerges as the main factor regulating the fasting-feeding induced changes in glycogen turnover during an OGTT. Such a turnover has been shown to occur in the liver, where it correlates with the glycogen content,[1,13] which is known to stimulate phosphorylase activity.[18] In contrast, muscle glycogen, even in the replete state, does not seem to undergo a significant turnover at rest during glucose and insulin infusion.[19] Thus, although skeletal muscle is probably an important site of glycogen storage during an OGTT,[20] the liver, through the fluctuations of its glycogen content, seems to be the central organ involved in the adaptation of whole body storage capacities to nutritional conditions at rest. This suggestion is in line with data showing that intraduodenal glucose administration to dogs after prior fasting stimulates net glycogen storage in the liver but not in muscle.[21] The slight stimulation of glycogen synthesis from oral glucose that might occur in liver and muscle after extending the fast might also be due to the reduced glycogen levels, which are known to modulate glycogen synthase activity.[18,22]

As regards the second factor able to modulate postglucose CHO fluxes, the elevated FFA concentrations and fat oxidation rates that persisted in previously fasted subjects during the OGTT might account for the decrease in glucose oxidation and therefore for the enhancement of oral glucose storage,[23] in an experimental setting in which glucose uptake is imposed. However, as already mentioned, the contribution of the latter mechanism to the stimulation of the net CHO balance is quantitatively small. More importantly, the increased FFA levels might contribute significantly to the inhibition of glycogen breakdown observed here, as documented in other studies.[16,24,25]

The third potential factor to be taken into consideration comprises the glycemic and insulinemic responses to glucose ingestion. In theory, their amplification by prior fasting might account for the inhibition of glycogenolysis and the small stimulation of glycogen synthesis from oral glucose observed under these conditions, but the metabolic effects of hyperinsulinemia should be attenuated or suppressed in the presence of the insulin resistance induced by fasting. Since glucagon levels were slightly more elevated after fasting (Table 2) this hormone should not be involved in the decreased glycogen breakdown. The observation (Fig. 1 and Table 2) that in the "fed" individuals, the changes in glycogen mobilization and synthesis occurred in the absence of any significant change in the glucose, insulin and glucagon responses underscores the minor importance of these circulatory factors in the "nutritional" regulation of the postprandial glycogen balance, in which the level of the glycogen stores seem to play the predominant role.

According to Fig 2 and Table 2, the relative contribution of the direct vs the indirect pathway in total glycogen synthesis increases with the degree of fasting. However, the physiologic significance of this observation is difficult to establish owing to the methodology used. This is because the indirect pathway is only active in the liver whereas the direct pathway is active in both muscle and liver, and these two tissues participate in unknown proportions in the whole body glycogen synthesis that is actually measured. Moreover, it has been demonstrated[26,27] that the relative importance of muscle and liver in whole body glycogen synthesis and, inside the liver, the relative importance of the 2 pathways may vary with time during the absorptive and early postabsorptive periods.

Group II

The experiments in which 33- and 100-g glucose loads were compared in overnight fasted subjects provided two pieces of information. First, they confirmed the results for group I. Indeed, unexpectedly large individual variations in basal CHO oxidation, presumably due to differences in the usual CHO consumption allowed us to confirm in each subgroup that the net CHO balance (Fig 3F) depends mainly on unlabeled glycogen oxidation (Fig 3E) and very little on oral glucose storage (Fig 3C). Second, these studies show (Table 3 and Fig 3) that increasing the oral load, and consequently the circulating glucose and insulin responses, stimulated the net CHO balance solely by augmenting oral glucose storage with no effect or even a slight nonsignificant increase in unlabeled glycogen mobilization. The fact that, in agreement with earlier studies,[28] increasing the glucose load only had a minor effect on the OGTT mean glucose concentration (+12%) but had a major effect on insulin levels (+171%, Table 3) might explain these observations. Recent in vivo studies in humans using [^{13}C]NMR spectroscopy indeed showed that hyperglycemia and hyperinsulinemia inhibit net hepatic glycogenolysis through distinct mechanisms: hyperglycemia acts by inhibiting the glycogen phosphorylase flux, whereas hyperinsulinemia activates the glycogen synthase flux.[29] These studies also demonstrated that these 2 fluxes are not necessarily coupled and coordinated in a reciprocal fashion as usually thought.

In conclusion, the dietary state of the individual and the size of the glucose load influence net postprandial CHO storage by different mechanisms. At a given glucose load, fasting increases net glycogen storage, mainly through a decrease in glycogen turnover, a decrease presumably mediated by the fall in hepatic glycogen content and possibly by increased fat oxidation. Opposite effects are induced by prior feeding. Whereas initial nutritional conditions influence the conversion of oral glucose to glycogen very little, raising the glucose load from 33 to 100 g in overnight fasted subjects augments net CHO storage solely by stimulating the rate of glycogen synthesis from oral glucose, but does not affect pre-existing glycogen mobilization. According to data from the literature,[29] the latter observation might be due to the fact that the main effect of increasing the glucose load is to enhance the insulinemic response to the glucose challenge rather than the glycemic response.

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